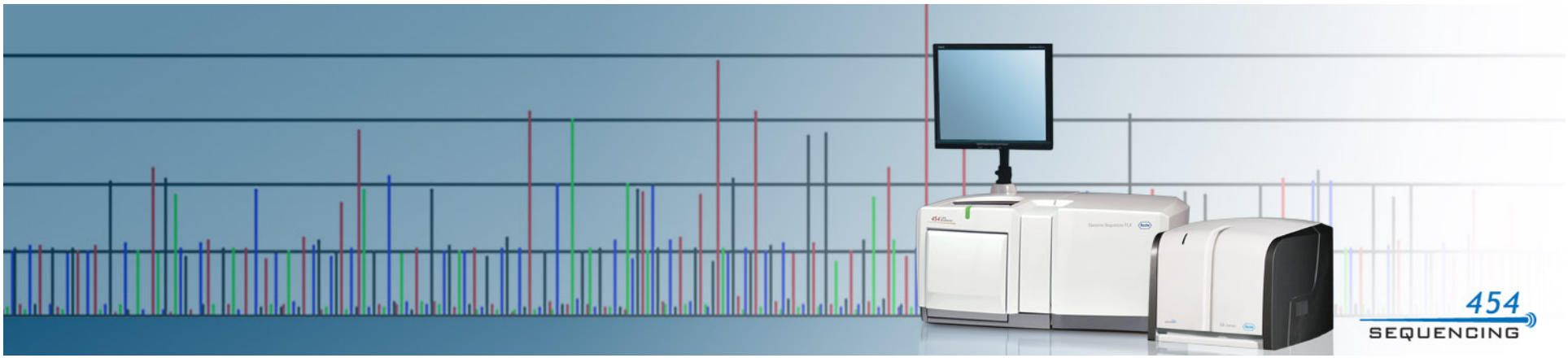
www.454.com

# 454 Sequencing
# The Road to the Future

*James R. Knight, PhD*

*05-Jun-2012*



# IMPORTANT NOTICE
## *Intended Use*

Unless explicitly stated otherwise, all Roche Applied Science and 454 Life Sciences products and services referenced in this presentation/document are intended for the following use:

**For life science research only.**

**Not for use in diagnostic procedures.**

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# 454 Sequencing Developments
## *Driving innovation across the product portfolio*

| Topic | Summary |
|---|---|
| 1 **GS Junior System** | - Extend read lengths for both amplicon and shotgun sequencing<br>- Automation for upfront workflow (lib prep & emPCR) |
| 2 **GS FLX+ System** | - Robustness and instrument improvements<br>- Optimize for long read sequencing |
| 3 **Assays** | - Launch virology assays for HIV/HCV/HBV resistance typing<br>- Expand HLA menu with registry typing assay |
| 4 **Future Technologies** | - Collaboration with DNA Electronics for development of semiconductor sequencing system<br>- Collaboration with IBM Research for innovative nanopore sequencing technology |

# Recent Developments

## Current Developments

## Future Developments*

* The following slides contains products in development, not available for sale

# Recent Developments

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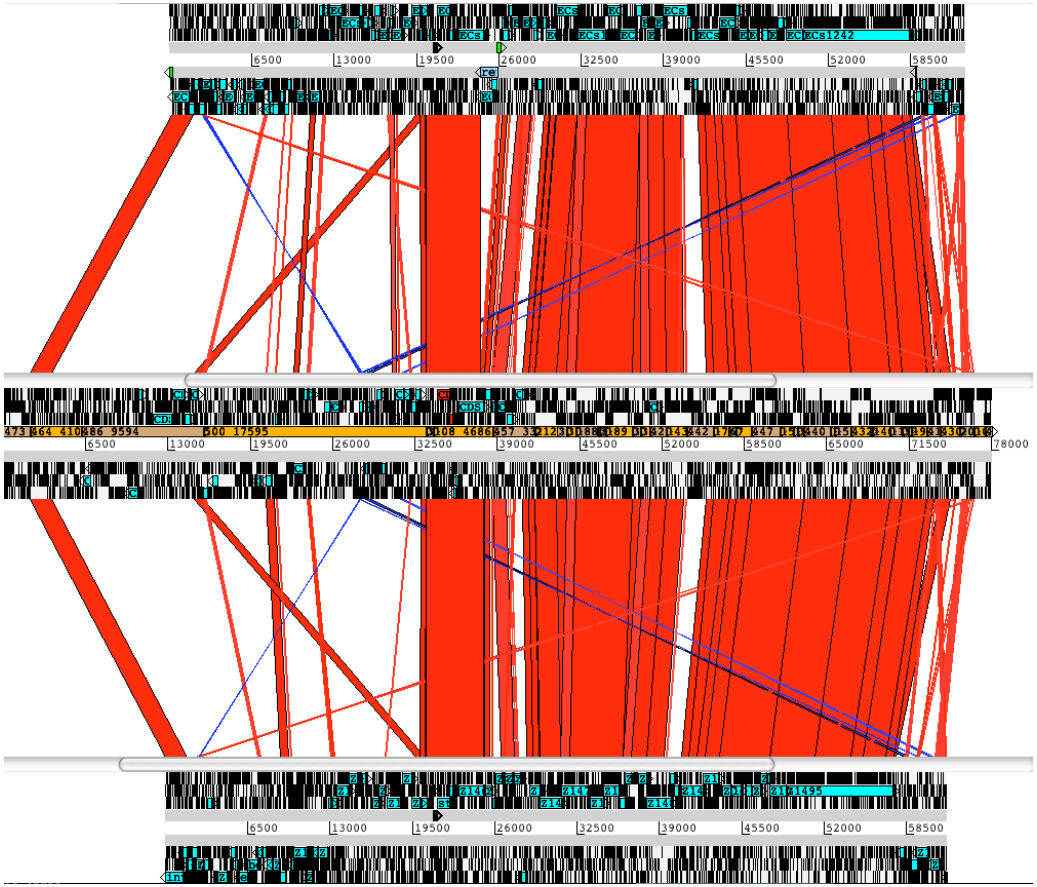
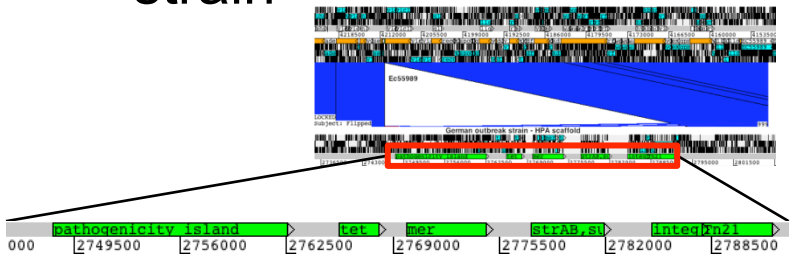
# Assembly of *E. coli* O104 Outbreak Genome

## *Day-by-day record on BacPathGenomics blog*



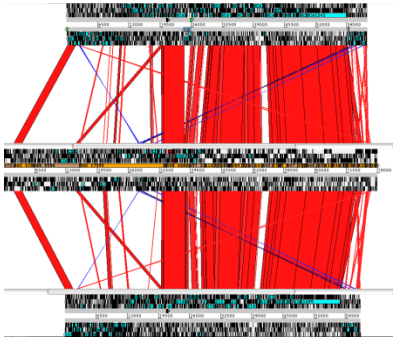
- June 9, 2011
  - Ion Torrent and Illumina data assemblies
  - ~450 contigs/scaffolds
  - Downstream analysis stuck at comparative genomes with known strain



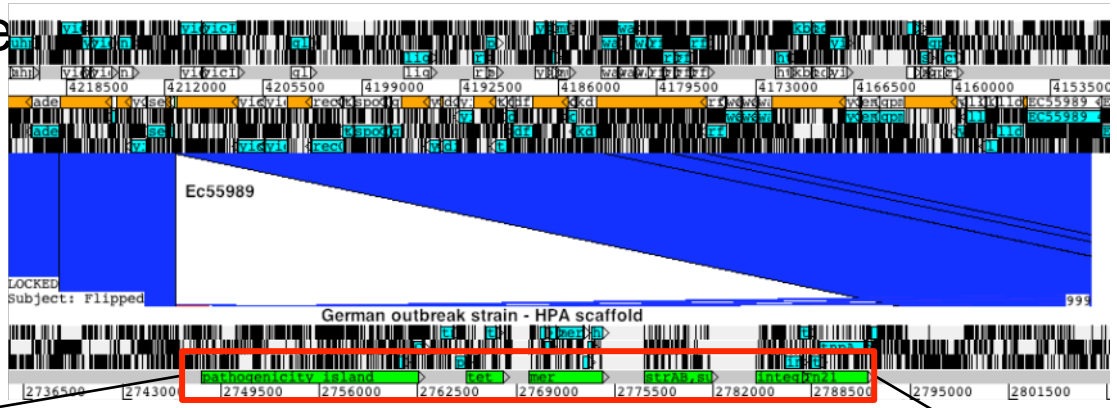

Source: Google search "bacpathgenomics june page 2"
http://bacpathgenomics.wordpress.com/2011/06/page/2/

# Assembly of *E. coli* O104 Outbreak Genome

## *Day-by-day record on BacPathGenomics blog*



- June 10, 2011
  - HPA assembly of 454 sequence, 13 scaffolds
  - Downstream analysis identifies inserted phage, predicts gene models and unlocks the

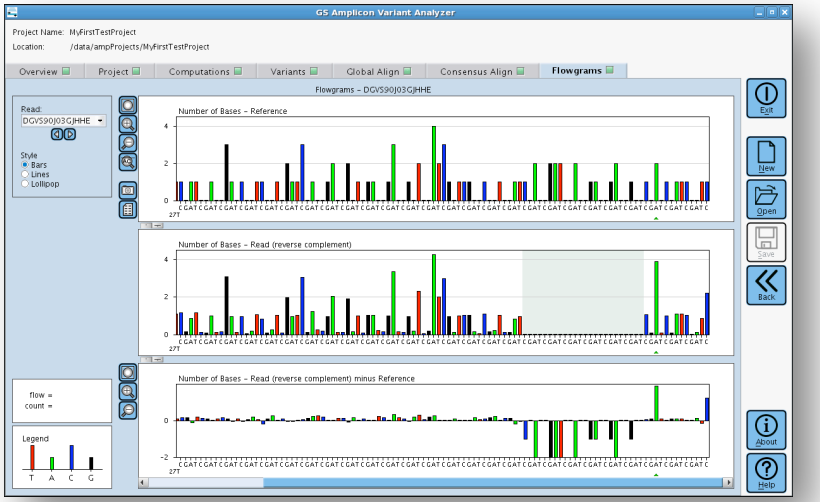




Source: Google search "bacpathgenomics june page 2"
http://bacpathgenomics.wordpress.com/2011/06/page/2/

# Released – GS Junior Software v2.7
## *What's New?*

- Software upgrade for improved sequencing robustness
- Functionality improvements to:
  - GS Amplicon Variant Analyzer (AVA)
  - GS *De Novo* Assembler
  - GS Reference Mapper
- Highlights include:
  - Detection of small insertions and deletions (1-2bp) in AVA
  - Simple de-multiplexing capabilities in Reference Mapper
  - New heterozygote function in Assembler




Screenshot of GS Amplicon Variant Analyzer software

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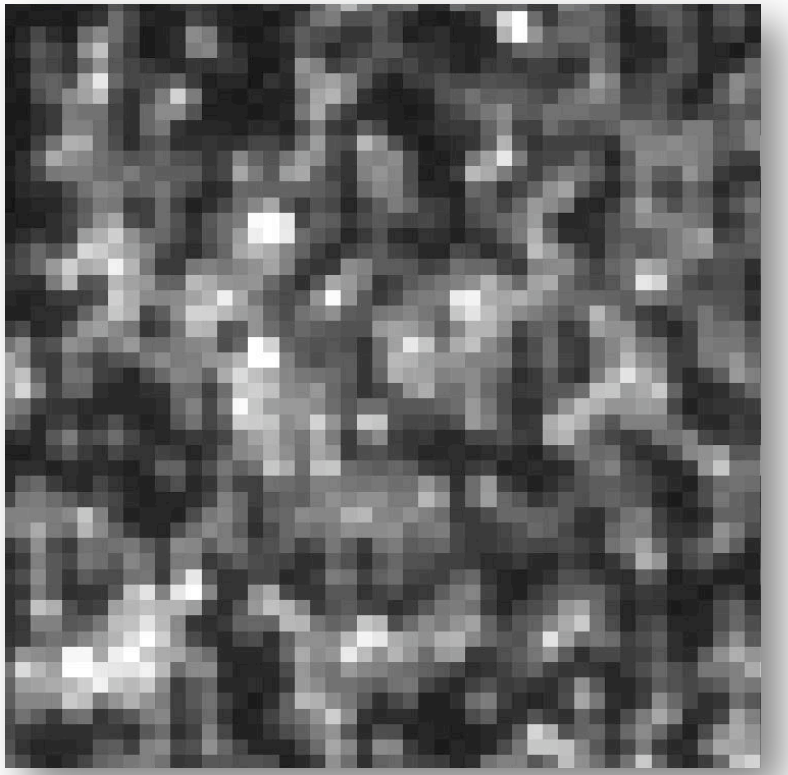
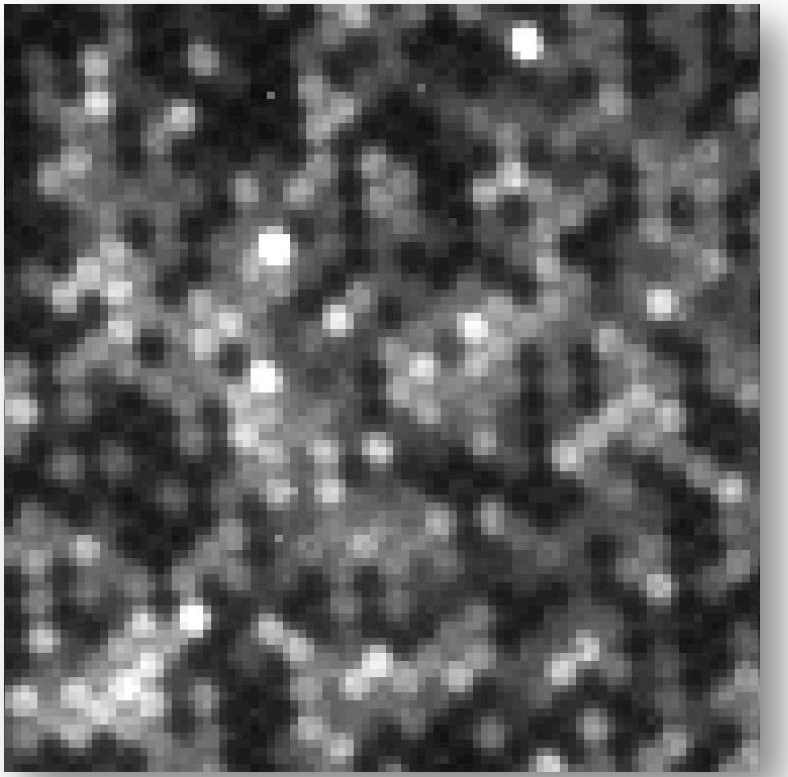
# Released – GS Junior Software v2.7

## *Software upgrade – better resolution of wells*

**v2.5 software**



**v2.7 software**



Improved resolution of wells results in better quality, more robust sequencing runs

No hardware changes required

# Released – GS Junior Software v2.7

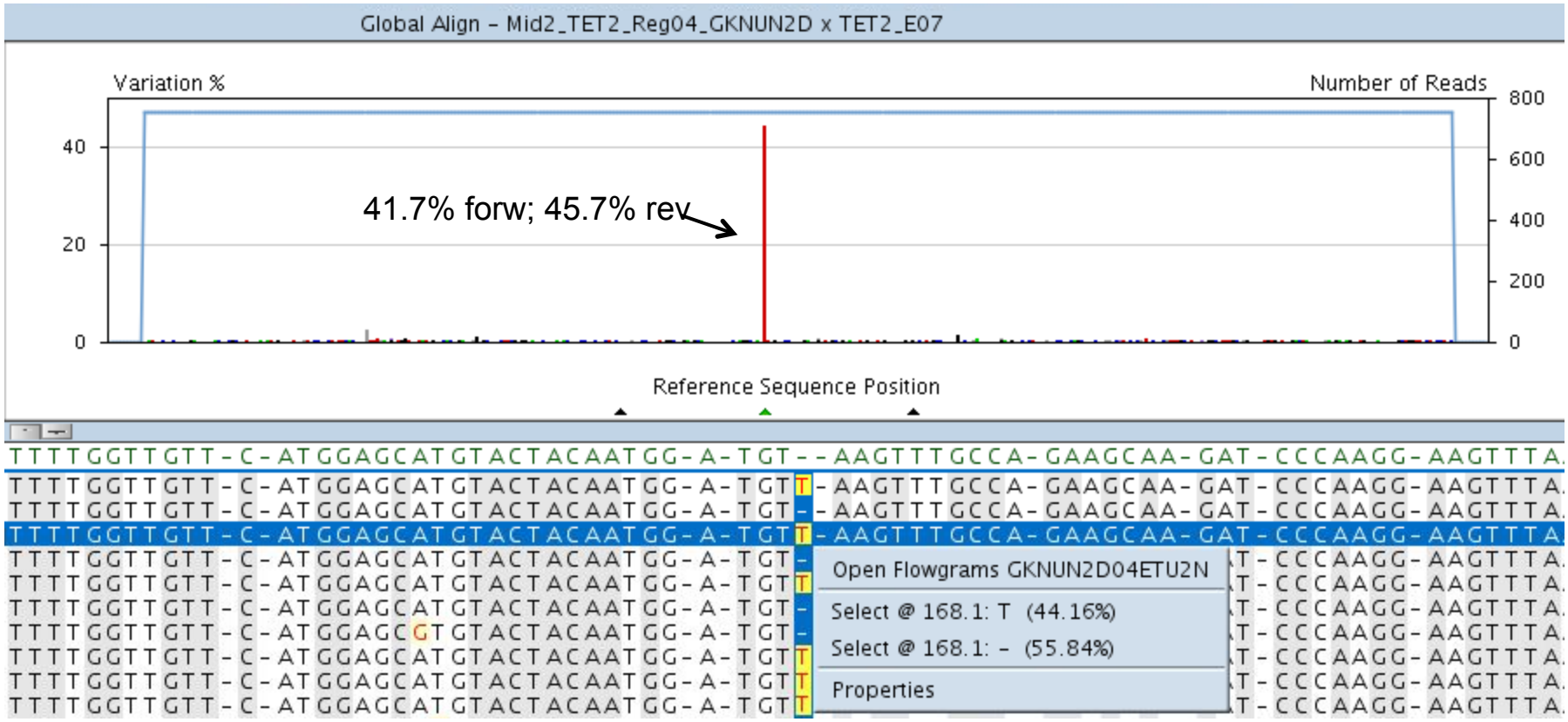
*Improved Assembly Software*

- Scaffold gap filling with –scaffold and –het
  - -scaffold enables repeat region gap filling
  - -het enables filling of local heterozygocity for diploid genomes
- Example 35MB fungal genome

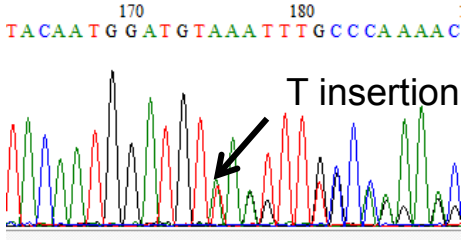
| scaffoldMetrics | v2.6 | v2.7_1454 |
|---|---|---|
| numberOfScaffolds | 14 | 13 |
| numberOfBases | 34,250,334 | 34,229,926 |
| avgScaffoldSize | 2,446,452 | 2,633,071 |
| N50ScaffoldSize | 3,438,441 | 3,436,801 |
| largestScaffoldSize | 3,865,581 | 4,485,751 |
| numberOfScaffoldContigs | **220** | **127** |
| numberOfScaffoldContigBases | 34,173,244 | 34,192,993 |
| avgScaffoldContigSize | **155,332** | **269,236** |
| N50ScaffoldContigSize | **395555, 27** | **639707, 18** |
| largestScaffoldContigSize | 1,148,004 | 2,011,999 |
| largeContigMetrics | | |
| numberOfContigs | 237 | 191 |
| numberOfBases | 34,184,671 | 34,205,596 |
| avgContigSize | **144,239** | **179,086** |
| N50ContigSize | **395,555** | **502,585** |
| largestContigSize | 1,148,004 | 1,161,175 |
| allContigMetrics | | |
| numberOfContigs | 470 | 431 |
| numberOfBases | 34,237,417 | 34,261,486 |

# Released – GS Junior Software v2.7

*Finding single base indels using AVA*




This mutation is the only real mutation in the 3 sample set not auto-detected by AVA v2.6 or earlier




T insertion confirmed by Sanger sequencing

# GS GType Assays for 454 Sequencing Systems
## *Available Now!*

### GS GType HLA MR & HR Primer Sets

- Sequence-based assays for high- and medium-resolution HLA typing
- For use on either the GS Junior or GS FLX System

### GS GType Leukemia Primer Sets (TET2/CBL/KRAS & RUNX1)

- Sequence-based assays for leukemia research
- For use on either the GS Junior or GS FLX System

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## Recent Developments

## Current Developments

## Future Developments*

* The following slides contains products in development, not available for sale

# GS FLX+ System - Current Developments
## *Software and instrument improvements*

- Improved upgrade procedure
- Reagent and instrument hardware improvements
  - Designed to increase robustness and reduce run-to-run variability
  - Rolling-out continuously as they become available
- Coming soon!  Software version 2.8
  - v2.7 functionality for FLX+ customers
  - Additional improvements to sequencing performance, particularly read length

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## Recent Developments

## Current Developments

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## Future Developments*

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# GS Junior Workflow – Future Developments
## *Integrated emPCR automation system*

Out of Scope | In Scope

### Current Automation

Library Prep → emPrep → emGen → emPCR → emBreaking → Enrichment → seqPrimers → Sequencing

***REM e System***

### Step 1: *Coming Soon*

**Library Prep** → emPrep → emGen → emPCR → **emBreaking** → **Enrichment** → **seqPrimers** → Sequencing

### Step 2: Beyond

**Library Prep** → emPrep → **emGen** → **emPCR** → **emBreaking** → **Enrichment** → **seqPrimers** → Sequencing

***Integrated emPCR automation system***

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# Collaboration with DNA Electronics
## *ISFET Sequencing Technology*

**Description**

- Collaboration to develop semiconductor-based sequencing system
- License DNAe's ISFET (ion-sensitive, field effect transistor) sequencing technology
- Enables detection of pH changes from nucleotide incorporation on semiconductor chip
- Builds on current 454 Sequencing portfolio by moving from optical to electrochemical detection
- Target applications in amplicon assays, gene panels, exome sequencing, RNA-seq, and whole genome sequencing

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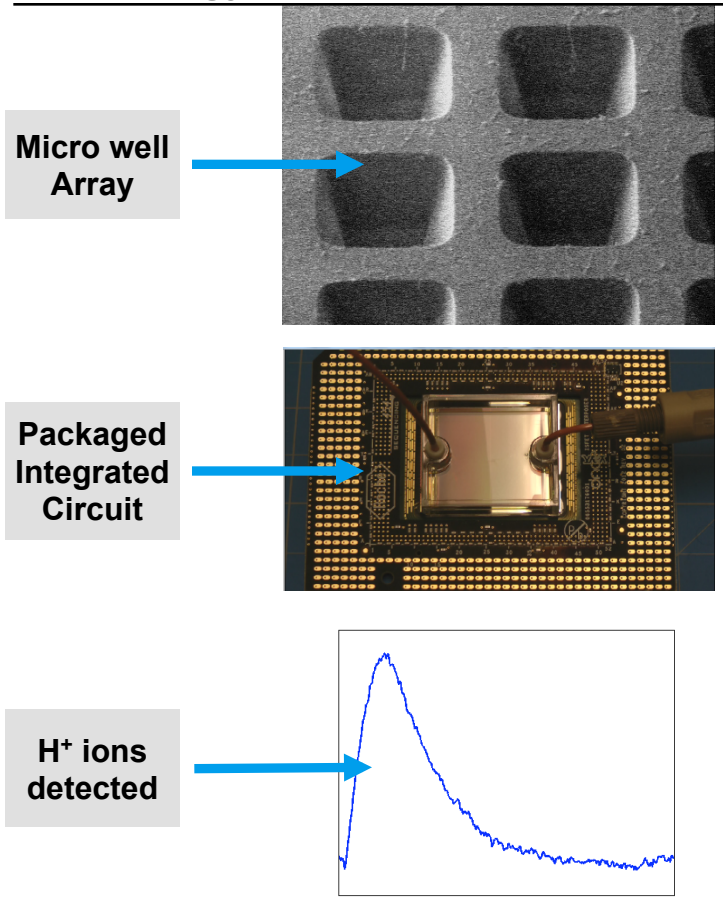
# Collaboration with DNAe: Technology
## *ISFETs enable label free pH Sensing on a Semiconductor Chip*

### Technology Concept




### Description

- **High-density array of micro-wells** on an integrated circuit. Each well holds a unique DNA template bead.
- Beneath each well is an **ion sensitive transistor**.
- The chip is sequentially flooded with one nucleotide after another. If the nucleotide that floods the chip is not a match to the DNA template, no change will be detected and no base will be called.
- If a nucleotide is incorporated into the growing strand complementary to the DNA template, a **$H^+$ ion** will be released which can be **detected** by the **ion sensitive transistor**.

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# Collaboration with IBM
## *Nanopore Sequencing Technology*

**Description**

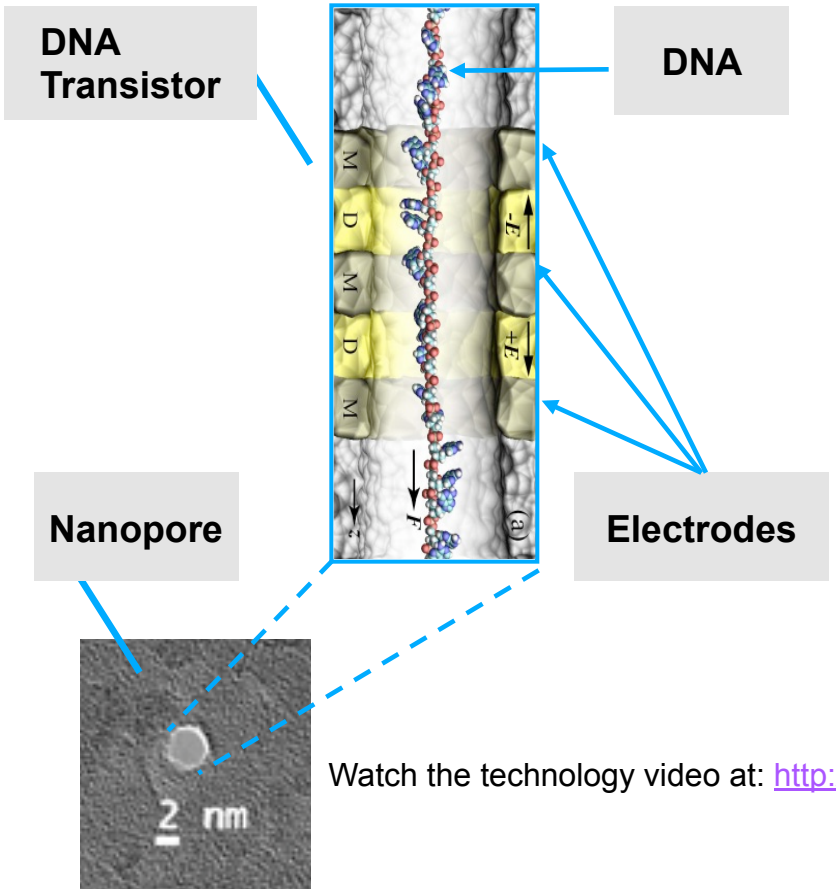
- Collaboration with IBM Research to develop nanopore-based sequencing system
- Technology based on IBM DNA Transistor concept
- Leverage IBM as a world leader in microelectronics, information technology and computational biology
- Access to world class prototype facilities for semi-conductor manufacturing and nanofabrication

**IBM**
T.J. Watson Research Center

# Collaboration with IBM: Technology Concept

## *Combining a nanopore transistor with a nucleotide sensing technology to identify DNA bases*

### Technology Concept




### Description

- **IBM DNA transistor concept** - series of alternating electrodes with insulator material in between, built into a silicon membrane that contains the **nanopores**.
- By changing the voltage applied to the electrodes, ssDNA can be made to move through the nanopore one base at a time (electrophoretic ratchet) in any direction.
- A **nucleotide sensing methodology** (recognition tunneling from ASU) is coupled to the DNA transistor to identify the bases in the DNA strand as it passes through the pore.

Watch the technology video at: http://www.youtube.com/watch?v=wvclP3GySUY

# 454 Sequencing Developments
## *Summary*

## Roche's Commitment to Sequencing

***Enhance*** – GS Junior & GS FLX+ Systems

***Develop*** - Assays

– ISFET Technology

***Innovate*** – Solid State Nanopore

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